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(57) Abstract

The present invention provides TRT antisense oligonucleotides, methods of detecting TRT, methods of diagnosing telomerase-related conditions, methods of diagnosing and providing a prognosis for cancer, and methods of treating telomerase-related conditions, including cancer.

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ANTISENSE COMPOSITIONS FOR DETECTING AND INHIBITING TELOMERASE REVERSE TRANSCRIPTASE

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation of U.S. Patent Application Serial No. 09/052,919, filed March 31, 1999, which is a continuation-in-part of U.S. Patent Application Serial Number 08/974,549, filed November 19, 1997, and a continuation-in-part of U.S. Patent Application Serial Number 08/974,584, filed November 19, 1997, both of which are continuation-in-part applications of U.S. Patent Application Serial Number 08/915,503, U.S. Patent Application Serial Number 08/912,951, and U.S. Patent Application Serial Number 08/911,312, all filed August 14, 1997, all three of which are continuation-in-part applications of U.S. Patent Application Serial Number 08/854,050, filed May 9, 1997, which is a continuation-in-part application of U.S. Patent Application Serial Number 08/851,843, filed May 6, 1997, which is a continuation-in-part application of U.S. Patent Application Serial Number 08/846,017, filed April 25, 1997, which is a continuation-in-part application of U.S. Patent Application Serial Number 08/844,419, filed April 18, 1996, which is a continuation-in-part application of U.S. Patent Application Serial Number 08/724,643, filed October 1, 1996. This application is also a continuation-in-part of Patent Convention Treaty Patent Application Serial No.: PCT/US97/17885 and to Patent Convention Treaty Patent Application Serial No.: PCT/US97/17618, both filed on October 1, 1997. Each of the aforementioned applications is explicitly incorporated herein by reference in its entirety and for all purposes.

FIELD OF THE INVENTION

The present invention provides TRT antisense oligonucleotides, methods of detecting TRT, methods of diagnosing telomerase-related conditions, methods of diagnosing and providing a prognosis for cancer, and methods of treating telomerase-related conditions, including cancer, with TRT antisense oligonucleotides.

BACKGROUND OF THE INVENTION

The following discussion is intended to introduce the field of the present invention to the reader. The citation of various references in this section should not be construed as an admission of prior invention.

It has long been recognized that complete replication of the ends of eukaryotic chromosomes requires specialized cell components (Watson, 1972, *Nature New Biol.*, 239:197; Olovnikov, 1973, *J. Theor. Biol.*, 41:181). Replication of a linear DNA strand by conventional DNA polymerases requires an RNA primer, and can proceed only 5' to 3'. When the RNA bound at the extreme 5' ends of eukaryotic chromosomal DNA strands is removed, a gap is introduced, leading to a progressive shortening of daughter strands with each round of replication. This shortening of telomeres, the protein-DNA structures physically located on the ends of chromosomes, is thought to account for the phenomenon of cellular senescence or aging of normal human somatic cells *in vitro* and *in vivo*. The length and integrity of telomeres is thus related to entry of a cell into a senescent stage (*i.e.*, loss of proliferative capacity), or the ability of a cell to escape senescence, *i.e.*, to become immortal. The maintenance of telomeres is a function of a telomere-specific DNA polymerase known as telomerase. Telomerase is a ribonucleoprotein (RNP) that uses a portion of its RNA moiety as a template for telomeric DNA synthesis (Morin, 1997, *Eur. J. Cancer* 33:750).

Consistent with the relationship of telomeres and telomerase to the proliferative capacity of a cell (*i.e.*, the ability of the cell to divide indefinitely), telomerase activity is detected in immortal cell lines and an extraordinarily diverse set of tumor tissues, but is not detected (i.e., was absent or below the assay threshold) in normal somatic cell cultures or normal tissues adjacent to a tumor (see, U.S. Patent Nos. 5,629,154; 5,489,508; 5,648,215; and 5,639,613; see also, Morin, 1989, *Cell* 59: 521; Shay and Bacchetti 1997, *Eur. J. Cancer* 33:787; Kim et al., 1994, *Science* 266:2011; Counter et al., 1992, *EMBO J.* 11:1921; Counter et al., 1994, *Proc. Natl. Acad. Sci. U.S.A.* 91, 2900; Counter et al., 1994, *J. Virol.* 68:3410). Moreover, a correlation between the level of telomerase activity in a tumor and the likely clinical outcome of the patient has been reported (e.g., U.S. Patent No. 5,639,613, *supra*; Langford et al., 1997,

Hum. Pathol. 28:416). Human telomerase is thus an ideal target for diagnosing and treating human diseases relating to cellular proliferation and senescence, such as cancer.

SUMMARY OF THE INVENTION

The present invention provides TRT antisense polynucleotides, which are useful for detecting, diagnosing, and treating telomerase-related conditions.

In one aspect, the present invention provides an isolated, synthetic, substantially pure, or recombinant polynucleotide having a sequence that is at least about ten nucleotides in length to at least about 100 nucleotides in length. This polynucleotide comprises a sequence that is substantially complementary or substantially identical to a contiguous sequence of an hTRT nucleic acid that has the nucleotide sequence of Figure 1.

In one aspect, the present invention provides an isolated, synthetic, substantially pure, or recombinant polynucleotide having a sequence that is at least about ten nucleotides in length to at least about 100 nucleotides in length. This polynucleotide comprises a sequence exactly complementary or identical to a contiguous sequence of a nucleic acid encoding the hTRT protein of Figure 2.

In one embodiment, the hTRT polynucleotide comprises a sequence that is exactly complementary or identical to a contiguous sequence of an hTRT nucleic acid having the nucleotide sequence of Figure 1.

In one embodiment, the polynucleotide is a DNA or an RNA. In one embodiment, the polynucleotide comprises one or more non-naturally occurring, synthetic nucleotides.

In one embodiment, the polynucleotide is identical to said contiguous sequence of a nucleic acid encoding the hTRT protein of Figure 1. In one embodiment, the polynucleotide is exactly complementary to said contiguous sequence of a nucleic acid encoding the hTRT protein of Figure 1.

In one embodiment, the polynucleotide is an antisense polynucleotide. In one embodiment, the polynucleotide is at least about 20 nucleotides in length to at least about 50 nucleotides in length.

In one embodiment, the polynucleotide inhibits telomerase activity by at least about 50% in transformed cells *ex vivo*, as compared to control cells that are not

treated with the polynucleotide. In one embodiment, the polynucleotide inhibits telomerase expression by at least about 50% *in vitro*, as compared to control expression reactions that lack the polynucleotide. In one embodiment, the polynucleotide is selected from the group consisting of PS-ODN number 3, 4, 7, 8, 16, 21, 25, 26, 27, 28, 29, 33, 40, 41, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 62, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 80, 81, 82, 83, 84, 85, 86, 87, 88, 93, 94, 96, 100, 112, 114, 130, 143, 144, 151, 152, 201, 202, 203, 208, 209, 210, 211, 212, 213, 230, 237, and 241.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 presents the nucleotide sequence of a cDNA encoding a naturally occurring human telomerase reverse transcriptase (hTRT) protein.

Fig. 2 presents the amino acid sequence of a naturally occurring, 1132-residue human telomerase reverse transcriptase (hTRT) protein.

Fig. 3 shows inhibition of hTRT expression *in vitro* by hTRT sequence-specific antisense phosphorothioate oligonucleotides (PS-ODN). Each bar in the graph represents the *in vitro* inhibitory activity of a specific oligonucleotide, numbered starting with PS-ODN #1. The PS-ODN are a series of 30-mers that span the hTRT mRNA and are offset one from the next by fifteen nucleotides. For example, ODN #1 corresponds to positions 16-35 of hTRT and is TCCCACGTGCGCAGCAGGACGCAGCGCTGC. ODN #2 corresponds to positions 31-60 and is GGCATCGCGGGGGTGGCCGGGGCCAG-GGCT, and so one to the end of the RNA (see the cDNA sequence of Figure 1, which represents an hTRT RNA sequence). The data are presented as a normalized percentage of the control with no added PS-ODN.

DETAILED DESCRIPTION

I. Introduction

Telomerase is a ribonucleoprotein complex (RNP) comprising an RNA component and a catalytic protein component. The catalytic protein component of human telomerase, hereinafter referred to as telomerase reverse transcriptase ("hTRT"), has been cloned, and protein, cDNA, and genomic sequences determined. See, e.g., Nakamura et al., 1997, *Science* 277:955, and copending U.S. Patent Applications Serial Nos. 08/912,951 and 08/974,549. The sequence of a full-length native hTRT has been

deposited in GenBank (Accession No. AF015950), and plasmid and phage vectors having hTRT coding sequences have been deposited with the American Type Culture Collection, Rockville, Maryland (accession numbers 209024, 209016, and 98505). The catalytic subunit protein of human telomerase has also been referred to as "hEST2" (Meyerson et al., 1997, *Cell* 90:785), "hTCS1" (Kilian et al., 1997, *Hum. Mol. Genet.* 6:2011), "TP2" (Harrington et al., 1997, *Genes Dev.* 11:3109), and "hTERT" (e.g., Greider, 1998, *Curr. Biol.* 8:R178-R181). The RNA component of human telomerase (hTR) has also been characterized (see U.S. Patent No. 5,583,016).

Human TRT is of extraordinary interest and value because, *inter alia*, telomerase activity in human cells and other mammalian cells correlates with cell proliferative capacity, cell immortality, and the development of a neoplastic phenotype. hTRT antisense polynucleotides, including the exemplary polynucleotides described herein, hybridize to and/or amplify naturally occurring hTRT genes or RNA. Such oligonucleotides are thus useful for diagnostic or prognostic applications to telomerase related conditions, including cancer. The hTRT antisense polynucleotides of the invention are also useful as therapeutic agents, e.g., antisense oligonucleotides, ribozymes, or triplex compositions, for inhibition of telomerase expression and activity (e.g., telomerase catalytic activity, *infra*).

The invention thus provides antisense oligonucleotide reagents, which can be used to detect expression of hTRT or reduce expression and activity of hTRT gene products *in vitro*, *ex vivo*, or *in vivo*. Administration of the antisense reagents of the invention to a target cell results in reduced telomerase activity, and is particularly useful for treatment of diseases characterized by high telomerase activity (e.g., cancers). Detection and inhibition of hTRT expression can be performed in a cell or cell extracts from a human, a mammal, a vertebrate, or other eukaryote.

The antisense polynucleotides of the invention are characterized by their ability to specifically hybridize to naturally occurring and synthetic hTRT nucleic acids, e.g., the hTRT gene, including any upstream, flanking, noncoding, and transcriptional control elements, hTRT pre-mRNA, mRNA, cDNA and the like. The hTRT antisense polynucleotides of the invention are typically at least 7-10 nucleotides in length to typically more 20 nucleotides up to about 100 nucleotides in length, preferably approximately 30 nucleotides in length. Such antisense oligonucleotides are used to detect

the presence of hTRT nucleic acid in a biological sample, for diagnosis and/or prognosis of telomerase related conditions, e.g., cancers of any of a wide variety of types, including solid tumors and leukemias, diseases of cell proliferation, disease resulting from cell senescence (particularly diseases of aging), immunological disorders, infertility, disease of immune dysfunction, etc.

The antisense polynucleotides of the invention also can be used to inhibit telomerase expression *in vitro*, to inhibit telomerase expression and activity in cells *ex vivo*, and can be used *in vivo* as therapeutic agents for the treatment of telomerase-related conditions listed above, including cancers of a wide variety of types (see, e.g., exemplary cancers listed in U.S. Patent Application Serial Number 08/974,549; and U.S. Patent Application Serial Number 08/974,584). In one embodiment of the invention, the antisense polynucleotides are 30 nucleotides in length, and have the ability to inhibit telomerase expression at least by 50% *in vitro* (see, e.g., the antisense oligonucleotides of Figure 3). In another embodiment of the invention, the antisense polynucleotides are 30 nucleotides in length, and have the ability to inhibit telomerase expression and activity at least 50% in transformed cells *ex vivo* (see, e.g., exemplary antisense hTRT oligonucleotides listed in Table 1).

II. Definitions

As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

As used herein, the terms "nucleic acid" and "polynucleotide" are used interchangeably. Use of the term "polynucleotide" includes oligonucleotides (i.e., short polynucleotides). This term also refers to deoxyribonucleotides, ribonucleotides, and naturally occurring variants, and can also refer to synthetic and/or non-naturally occurring nucleic acids (i.e., comprising nucleic acid analogues or modified backbone residues or linkages), such as, for example and without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), and the like, as described herein.

As used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of approximately 7 nucleotides or greater in length, and up to as many as approximately 100 nucleotides in length, which can be used as a primer, probe or

amplimer. Oligonucleotides are often between about 10 and about 50 nucleotides in length, more often between about 14 and about 35 nucleotides, very often between about 15 and about 30 nucleotides, and the terms oligonucleotides or oligomers can also refer to synthetic and/or non-naturally occurring nucleic acids (i.e., comprising nucleic acid analogues or modified backbone residues or linkages).

A polynucleotide "specifically hybridizes" or "specifically binds" to a target polynucleotide if the polynucleotide hybridizes to the target under stringent conditions. As used herein, "stringent hybridization conditions" or "stringency" refers to conditions in a range from about 5°C to about 20°C or 25°C below the melting temperature (T_m) of the target sequence and a probe with exactly or nearly exactly complementarity to the target. As used herein, the melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half-dissociated into single strands. Methods for calculating the T_m of nucleic acids are well known in the art (see, e.g., Berger and Kimmel (1987) *Methods in Enzymology*, Vol. 152: *Guide to Molecular Cloning Techniques*, San Diego: Academic Press, Inc.; Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Vols. 1-3, Cold Spring Harbor Laboratory hereinafter, "Sambrook"); and *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds. through and including the 1997 supplement), incorporated herein by reference). As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\% G + C)$, when a nucleic acid is in aqueous solution at 1 M NaCl (see e.g., Anderson and Young, *Quantitative Filter Hybridization* in *Nucleic Acid Hybridization* (1985)). Other references include more sophisticated computations, which take structural as well as sequence characteristics into account for the calculation of T_m. The melting temperature of a hybrid (and thus the conditions for stringent hybridization) is affected by various factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, and the like), and the concentration of salts and other components (e.g., the presence or absence of formamide, dextran sulfate, polyethylene glycol). The effects of these factors are well known and are discussed in standard references in the art, e.g., Sambrook, *supra* and Ausubel et al. *supra*. Typically, stringent hybridization conditions are salt concentrations less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion at pH 7.0 to 8.3, and temperatures at least

about 30°C for short nucleic acids (e.g., 7 to 50 nucleotides) and at least about 60°C for long nucleic acids (e.g., greater than 50 nucleotides). As noted, stringent conditions may also be achieved with the addition of destabilizing agents such as formamide, in which case lower temperatures may be employed.

An "identical" polynucleotide refers to a polynucleotide that has the same sequence as the reference nucleotide subsequence to which the polynucleotide is being compared. An "exactly complementary" polynucleotide refers to a polynucleotide whose complement has the same sequence as the reference nucleotide subsequence to which the polynucleotide is being compared.

A "substantially complementary" polynucleotide and a "substantially identical" polynucleotide have the ability to specifically hybridize to a reference gene, DNA, cDNA, or mRNA, e.g., the hTRT nucleotide sequence of Figure 1 and its exact complement.

An "antisense" polynucleotide is a polynucleotide that is substantially complementary to a target polynucleotide and has the ability to specifically hybridize to the target polynucleotide.

A "telomerase-related condition" refers to a diseases and disease conditions in a patient and/or a cell, characterized by under- or over-expression of telomerase or hTRT gene products. In addition to cancer, which is characterized by over-expression of telomerase, such conditions include diseases of cell proliferation, e.g., hyperplasias, disease resulting from cell senescence (particularly diseases of aging), immunological disorders, infertility, etc.

As used herein, "isolated," when referring to a molecule or composition, such as, for example, an oligonucleotide, means that the molecule or composition is separated from at least one other compound, such as other oligonucleotides or other contaminants with which it is associated *in vivo* or in its naturally occurring state or synthetic state. An isolated composition can also be substantially pure.

A "synthetic" oligonucleotide refers to a polynucleotide synthesized using *in vitro* chemical methods, e.g., by using a machine that synthesizes polynucleotides using the phosphodiester method, the diethylphosphoramidite method, the phosphotriester methods, the solid support method, and other methods known to those skilled in the art.

As used herein, "recombinant" refers to a polynucleotide synthesized or otherwise manipulated *in vitro* (e.g., "recombinant polynucleotide"), to methods of using recombinant polynucleotides to produce gene products in cells or other biological systems, or to a polypeptide ("recombinant protein") encoded by a recombinant polynucleotide.

As used herein, the term "substantially pure," or "substantially purified," when referring to a composition comprising a specified reagent, such as an oligonucleotide, means that the specified reagent is at least about 75%, or at least about 90%, or at least about 95%, or at least about 99% or more of the composition (not including, e.g., solvent or buffer). Thus, for example, an antisense oligonucleotide preparation that specifically binds an hTRT gene or mRNA is substantially purified.

"TRT" activity refers to one or more of the activities found in naturally-occurring full-length TRT proteins. These activities include "telomerase catalytic activity" (the ability to extend a DNA primer that functions as a telomerase substrate by adding a partial, one, or more than one repeat of a sequence, e.g., TTAGGG, encoded by a template nucleic acid, e.g., hTR), "telomerase conventional reverse transcriptase activity" (see Morin, 1997, *supra*, and Spence et al., 1995, *Science* 267:988); "nucleolytic activity" (see Morin, 1997, *supra*; Collins and Grieder, 1993, *Genes and Development* 7:1364; Joyce and Steitz, 1987, *Trends Biochem. Sci.* 12:288); "primer (telomere) binding activity" (see, Morin, 1997, *supra*; Collins et al., 1995, *Cell* 81:677; Harrington et al., 1995, *J. Biol. Chem.* 270:8893); "dNTP binding activity" (Morin, 1997, *supra*; Spence et al., *supra)*; and "RNA (e.g., hTR) binding activity" (see Morin, 1997, *supra*; Harrington et al., 1997, *Science* 275:973; Collins et al., 1995, *Cell* 81:677).

"TRT" refers to telomerase reverse transcriptase protein, and "hTRT" refers to human telomerase reverse transcriptase protein.

The term "hTRT" is intended to refer to alleles, conservatively modified variants, polymorphic variants, and interspecies homologues of hTRT encoded by nucleic acids that specifically hybridize to the hTRT nucleic acid sequence provided in Figure 1.

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids that encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence,

to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art (see, e.g., Creighton (1984) *Proteins*, W.H. Freeman and Company.

III. How to make antisense polynucleotides

As described herein, the present invention provides antisense polynucleotides, which have the ability to specifically hybridize to hTRT. Without intending to be limited to any particular mechanism, it is believed that antisense oligonucleotides bind to, and interfere with the translation of, the sense hTRT mRNA. Alternatively, the antisense molecule may render the hTRT mRNA susceptible to nuclease digestion, interfere with transcription, interfere with processing, localization or otherwise with RNA precursors ("pre-mRNA"), repress transcription of mRNA from the hTRT gene, or act through some other mechanism. However, the particular mechanism by which the antisense molecule reduces hTRT expression is not critical.

Generally, to assure specific hybridization, the antisense sequence is substantially complementary to the target hTRT mRNA sequence. In certain

embodiments, the antisense sequence is exactly complementary to the target sequence. The antisense polynucleotides may also include, however, nucleotide substitutions, additions, deletions, transitions, transpositions, or modifications, or other nucleic acid sequences or non-nucleic acid moieties so long as specific binding to the relevant target sequence corresponding to hTRT RNA or its gene is retained as a functional property of the polynucleotide.

In one embodiment, the antisense sequence is complementary to relatively accessible sequences of the hTRT mRNA (e.g., relatively devoid of secondary structure). These sequences can be determined by analyzing predicted RNA secondary structures using, for example, the MFOLD program (Genetics Computer Group, Madison WI) and testing *in vitro* or *in vivo* as is known in the art. Figure 3 and TAble 1 show examples of oligonucleotides that are useful in cells for antisense suppression of hTRT function and are capable of hybridizing to hTRT (i.e., are substantially complementary to hTRT). Another useful method for identifying effective antisense compositions uses combinatorial arrays of oligonucleotides (see, e.g., Milner et al., 1997, *Nature Biotechnology* 15:537).

A. Triplex-forming antisense polynucleotides

As one embodiment of the antisense molecules described herein, the present invention provides polynucleotides that bind to double-stranded or duplex hTRT nucleic acids (e.g., in a folded region of the hTRT RNA or in the hTRT gene), forming a triple helix-containing, or "triplex" nucleic acid. Triple helix formation results in inhibition of hTRT expression by, for example, preventing transcription of the hTRT gene, thus reducing or eliminating telomerase activity in a cell. Without intending to be bound by any particular mechanism, it is believed that triple helix pairing compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules to occur.

Triplex oligo- and polynucleotides of the invention are constructed using the base-pairing rules of triple helix formation (see, e.g., Cheng et al., 1988, *J. Biol. Chem.* 263: 15110; Ferrin and Camerini-Otero, 1991, *Science* 354:1494; Ramdas et al., 1989, *J. Biol. Chem.* 264:17395; Strobel et al., 1991, *Science* 254:1639; and Rigas et al., 1986, *Proc. Natl. Acad. Sci. U.S.A.* 83: 9591; each of which is incorporated herein by reference) and the hTRT mRNA and/or gene sequence. Typically, the triplex-forming

oligonucleotides of the invention comprise a specific sequence of from about 10 to at least about 25 nucleotides or longer "complementary" to a specific sequence in the hTRT RNA or gene (i.e., large enough to form a stable triple helix, but small enough, depending on the mode of delivery, to administer *in vivo*, if desired). In this context, "complementary" means able to form a stable triple helix. In one embodiment, oligonucleotides are designed to bind specifically to the regulatory regions of the hTRT gene (e.g., the hTRT 5'-flanking sequence, promoters, and enhancers) or to the transcription initiation site, (e.g., between -10 and +10 from the transcription initiation site). For a review of recent therapeutic advances using triplex DNA, see Gee et al., *in* Huber and Carr, 1994, *Molecular and Immunologic Approaches*, Futura Publishing Co, Mt Kisco NY and Rininsland et al., 1997, *Proc. Natl. Acad. Sci. USA* 94:5854, which are both incorporated herein by reference.

B. Ribozymes

In another embodiment, the present invention provides ribozymes useful for inhibition of hTRT telomerase activity. The ribozymes of the invention bind and enzymatically cleave and inactivate hTRT mRNA. Useful ribozymes can comprise 5'- and 3'-terminal sequences complementary to the hTRT mRNA and can be engineered by one of skill on the basis of the hTRT mRNA sequence disclosed herein (see PCT publication WO 93/23572, *supra)*. Ribozymes of the invention include those having characteristics of group I intron ribozymes (Cech, 1995, *Biotechnology* 13:323) and others of hammerhead ribozymes (Edgington, 1992, *Biotechnology* 10:256).

Ribozymes of the invention include those having cleavage sites such as GUA, GUU and GUC. Other optimum cleavage sites for ribozyme-mediated inhibition of telomerase activity in accordance with the present invention include those described in PCT publications WO 94/02595 and WO 93/23569, both incorporated herein by reference. Short RNA oligonucleotides between 15 and 20 ribonucleotides in length corresponding to the region of the target hTRT gene containing the cleavage site can be evaluated for secondary structural features that may render the oligonucleotide more desirable. The suitability of cleavage sites may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection

assays, or by testing for *in vitro* ribozyme activity in accordance with standard procedures known in the art.

As described by Hu et al., PCT publication WO 94/03596, incorporated herein by reference, antisense and ribozyme functions can be combined in a single oligonucleotide. Moreover, ribozymes can comprise one or more modified nucleotides or modified linkages between nucleotides, as described above in conjunction with the description of illustrative antisense oligonucleotides of the invention.

C. Synthesis of antisense polynucleotides

The antisense nucleic acids (DNA, RNA, modified, analogues, and the like) can be made using any suitable method for producing a nucleic acid, such as the chemical synthesis and recombinant methods disclosed herein and known to one of skill in the art. In one embodiment, for example, antisense RNA molecules of the invention may be prepared by *de novo* chemical synthesis or by cloning. For example, an antisense RNA that hybridizes to hTRT mRNA can be made by inserting (ligating) an hTRT DNA sequence in reverse orientation operably linked to a promoter in a vector (e.g., plasmid). Provided that the promoter and, preferably termination and polyadenylation signals, are properly positioned, the strand of the inserted sequence corresponding to the noncoding strand will be transcribed and act as an antisense oligonucleotide of the invention.

The present invention also provides hTRT antisense polynucleotides (RNA, DNA or modified) that can be produced by direct chemical synthesis. Chemical synthesis is generally preferred for the production of oligonucleotides or for oligonucleotides and polynucleotides containing nonstandard nucleotides (e.g., probes, primers and antisense oligonucleotides). Direct chemical synthesis of nucleic acids can be accomplished by methods known in the art, such as the phosphotriester method of Narang et al., 1979, *Meth. Enzymol.* 68:90; the phosphodiester method of Brown et al., *Meth. Enzymol.* 68:109 (1979); the diethylphosphoramidite method of Beaucage et al., *Tetra. Lett.*, 22:1859 (1981); and the solid support method of U.S. Patent No. 4,458,066.

Chemical synthesis typically produces a single stranded oligonucleotide, which may be converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase and an oligonucleotide primer using the single strand as a template. One of skill will recognize

that while chemical synthesis of DNA is often limited to sequences of about 100 or 150 bases, longer sequences may be obtained by the ligation of shorter sequences or by more elaborate synthetic methods.

It will be appreciated that the hTRT polynucleotides and oligonucleotides of the invention can be made using nonstandard bases (e.g., other than adenine, cytidine, guanine, thymine, and uridine) or nonstandard backbone structures to provides desirable properties (e.g., increased nuclease-resistance, tighter-binding, stability or a desired T_M). Techniques for rendering oligonucleotides nuclease-resistant include those described in PCT publication WO 94/12633. A wide variety of useful modified oligonucleotides may be produced, including oligonucleotides having a peptide-nucleic acid (PNA) backbone (Nielsen et al., 1991, *Science* 254:1497) or incorporating 2'-O-methyl ribonucleotides, phosphorothioate nucleotides, methyl phosphonate nucleotides, phosphotriester nucleotides, phosphorothioate nucleotides, phosphoramidates. Still other useful oligonucleotides may contain alkyl and halogen-substituted sugar moieties comprising one of the following at the 2' position: OH, SH, SCH_3, F, OCN, OCH_3OCH_3, $OCH_3O(CH_2)_nCH_3$, $O(CH_2)_nNH_2$ or $O(CH_2)_nCH_3$, where n is from 1 to about 10; C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF_3; OCF_3; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; $SOCH_3$; SO_2CH_3; ONO_2; NO_2; N_3; NH_2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a cholesteryl group; a folate group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. Folate, cholesterol or other groups that facilitate oligonucleotide uptake, such as lipid analogs, may be conjugated directly or via a linker at the 2' position of any nucleoside or at the 3' or 5' position of the 3'-terminal or 5'-terminal nucleoside, respectively. One or more such conjugates may be used. Oligonucleotides may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group. Other embodiments may include at least one modified base form or "universal base" such as inosine, or inclusion of other nonstandard bases such as queosine and wybutosine as well as acetyl-, methyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

The invention further provides oligonucleotides having backbone analogues such as phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, phosphoramidate, alkyl phosphotriester, sulfamate, 3'-thioacetal, methylene(methylimino), 3'-N-carbamate, morpholino carbamate, chiral-methyl phosphonates, nucleotides with short chain alkyl or cycloalkyl intersugar linkages, short chain heteroatomic or heterocyclic intersugar ("backbone") linkages, or CH_2-NH-O-CH_2, CH_2-N(CH_3)-OCH_2, CH_2-O-N(CH_3)-CH_2, CH_2-N(CH_3)-N(CH_3)-CH_2 and O-N(CH_3)-CH_2-CH_2 backbones (where phosphodiester is O-P-O-CH_2), or mixtures of the same. Also useful are oligonucleotides having morpholino backbone structures (U.S. Patent No. 5,034,506).

Useful references include *Oligonucleotides and Analogues, A Practical Approach*, edited by F. Eckstein, IRL Press at Oxford University Press (1991); *Antisense Strategies, Annals of the New York Academy of Sciences*, Volume 600, Eds. Baserga and Denhardt (NYAS 1992); Milligan et al., 9 July 1993, *J. Med. Chem.* 36(14):1923-1937; *Antisense Research and Applications* (1993, CRC Press), in its entirety and specifically Chapter 15, by Sanghvi, entitled "Heterocyclic base modifications in nucleic acids and their applications in antisense oligonucleotides;" and *Antisense Therapeutics*, ed. Sudhir Agrawal (Humana Press, Totowa, New Jersey, 1996).

D. Labeled antisense oligonucleotides

It is often useful to label the antisense polynucleotides of the invention, for example, when the hTRT polynucleotides are to be used for detection of hTRT expression, and for diagnosis and prognosis of telomerase related conditions. The labels may be incorporated by any of a number of means well known to those of skill in the art. Suitable labels are any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, ^{35}S, fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin-streptavadin, digoxigenin, haptens and proteins for which antisera or monoclonal antibodies are available, or nucleic acid molecules with a sequence complementary to a target. The label often generates a measurable signal, such as radioactivity, that can be used to quantitate the amount of bound detectable moiety.

The label can be incorporated in or attached to a polynucleotide either covalently, or through ionic, van der Waals or hydrogen bonds, e.g., incorporation of

radioactive nucleotides, or biotinylated nucleotides that are recognized by streptavadin. The detectable moiety may be directly or indirectly detectable. Indirect detection can involve the binding of a second directly or indirectly detectable moiety to the detectable moiety. For example, the detectable moiety can be the ligand of a binding partner, such as biotin, which is a binding partner for streptavadin, or a nucleotide sequence, which is the binding partner for a complementary sequence, to which it can specifically hybridize. The binding partner may itself be directly detectable, for example, an antibody may be itself labeled with a fluorescent molecule. The binding partner also may be indirectly detectable, for example, a nucleic acid having a complementary nucleotide sequence can be a part of a branched DNA molecule that is in turn detectable through hybridization with other labeled nucleic acid molecules.

IV. Exemplary antisense polynucleotides

A series of 30-mer antisense oligonucleotides, which span the entire hTRT sequence, are exemplary embodiments of the present invention (see Figure 3). These oligonucleotides were systematically assayed for the ability to inhibit hTRT expression *in vitro*. The results of the experiment are presented in Figure 3 (see also Example I). Any suitable series of hTRT antisense oligonucleotides can be tested in a similar fashion. For example, a series of 20-mer antisense oligonucleotides, offset one from the next by 10 nucleotides can be synthesized and tested in the same manner. A series of 25-mer, 35-mer, or 15-mer oligonucleotides can be examined in the same manner.

Selected oligonucleotides from the series of Figure 3 were then tested *ex vivo* for their ability to inhibit hTRT expression in tumor cells (see Example II). The hTRT antisense oligonucleotides active for inhibiting telomerase activity *ex vivo* in tumor cells were than assayed for their long term cell culture effects on hTRT expression, telomerase activity, telomere dynamics, and cell proliferation (see Example II). The oligonucleotides of Table I represent exemplary oligonucleotides that inhibited telomerase activity *ex vivo*.

TABLE I

hTRT AS 30-mers: Telomerase Activity Relative to Untreated Cells

PS-ODN#	Position (3'-5')	5'-AS sequence-3'
3	31-60	GGCATCGCGGGGGTGGCCGGGGCCAGGGCT
4	46-75	CAGCGGGGAGCGCGCGGCATCGCGGGGGTG
7	91-120	AGCACCTCGCGGTAGTGGCTGCGCAGCAGG
8	106-135	AACGTGGCCAGCGGCAGCACCTCGCGGTAG
16	226-255	GCGGGGGGCGGCCGTGCGTCCCAGGGCACG
21	301-330	CCGCGCTCGCACAGCCTCTGCAGCACTCGG
25	361-390	GGGGGGCCCCCGCGGGCCCCGTCCAGCAGC
26	376-405	GTGGTGAAGGCCTCGGGGGGGCCCCCGCGG
27	391-420	TAGCTGCGCACGCTGGTGGTGAAGGCCTCG
28	406-435	ACCGTGTTGGGCAGGTAGCTGCGCACGCTG
29	421-450	CGCAGTGCGTCGGTCACCGTGTTGGGCAGG
33	481-510	AGGTGAACCAGCACGTCGTCGCCCACGCGG
40	586-615	GGGGGCCGGGCCTGAGTGGCAGCGCCGAGC
41	601-630	CCACTAGCGTGTGGCGGGGGCCGGGCCTGA
43	631-660	GCCCGTTCGCATCCCAGACGCCTTCGGGGT
44	646-675	ACGCTATGGTTCCAGGCCCGTTCGCATCCC
45	661-690	ACCCCGGCCTCCCTGACGCTATGGTTCCAG
46	676-705	GGCAGGCCCAGGGGGACCCCGGCCTCCCTG
47	691-720	CTCGCACCCGGGGCTGGCAGGCCCAGGGGG

PS-ODN#	Position (3'-5')	5'-AS sequence-3'
48	706-735	CTGCCCCCGCGCCTCCTCGCACCCGGGGCT
49	721-750	AGACTTCGGCTGGCACTGCCCCCGCGCCTC
50	736-765	CTCTTGGGCAACGGCAGACTTCGGCTGGCA
51	751-780	GCGCCACGCCTGGGCCTCTTGGGCAACGGC
52	766-795	TCCGGCTCAGGGGCAGCGCCACGCCTGGGC
53	781-810	CCAACGGGCGTCCGCTCCGGCTCAGGGGCA
54	796-825	GCCCAGGACCCCTGCCCAACGGGCGTCCGC
62	916-945	GGGTGGGAGTGGCGCGTGCCAGAGAGCGCA
68	1006-1035	TCGGCGTACACCGGGGGACAAGGCGTGTCC
69	1021-1050	AGGAAGTGCTTGGTCTCGGCGTACACCGGG
70	1036-1065	TCGCCTGAGGAGTAGAGGAAGTGCTTGGTC
71	1051-1080	CGCAGCTGCTCCTTGTCGCCTGAGGAGTAG
72	1066-1095	AGTAGGAAGGAGGGCCGCAGCTGCTCCTTG
73	1081-1110	GGCCTCAGAGAGCTGAGTAGGAAGGAGGGC
74	1096-1125	GCGCCAGTCAGGCTGGGCCTCAGAGAGCTG
75	1111-1140	TCCACGAGCCTCCGAGCGCCAGTCAGGCTG
76	1126-1155	CCCAGAAAGATGGTCTCCACGAGCCTCCGA
77	1141-1170	ATCCAGGGCCTGGAACCCAGAAAGATGGTC
80	1186-1215	CAGTAGCGCTGGGGCAGGCGGGGCAACCTG
81	1201-1230	AGGGGCCGCATTTGCCAGTAGCGCTGGGGC
82	1216-1245	AGCAGCTCCAGAAACAGGGGCCGCATTTGC
83	1231-1260	TGCGCGTGGTTCCCAAGCAGCTCCAGAAAC

PS-ODN#	Position (3'-5')	5'-AS sequence-3'
84	1246-1275	ACCCCGTAGGGGCACTGCGCGTGGTTCCCA
85	1261-1290	TGCGTCTTGAGGAGCACCCCGTAGGGGCAC
86	1276-1305	GCTCGCAGCGGGCAGTGCGTCTTGAGGAGC
87	1291-1320	GCTGGGGTGACCGCAGCTCGCAGCGGGCAG
88	1306-1335	GCACAGACACCGGCTGCTGGGGTGACCGCA
93	1381-1410	AGCAGCTGCACCAGGCGACGGGGGTCTGTG
94	1396-1425	CTGCTGTGCTGGCGGAGCAGCTGCACCAGG
96	1426-1455	GCCCGCACGAAGCCGTACACCTGCCAGGGG
100	1486-1515	AAGCGGCGTTCGTTGTGCCTGGAGCCCCAG
112	1666-1695	CAGTGCAGGAACTTGGCCAGGATCTCCTCA
114	1696-1725	AGCAGCTCGACGACGTACACACTCATCAGC
130	1936-1965	TCCATGTTCACAATCGGCCGCAGCCCGTCA
143	2131-2160	GGGTCCTGGGCCCGCACACGCAGCACGAAG
144	2146-2175	TACAGCTCAGGCGGCGGGTCCTGGGCCCGC
151	2251-2280	CGCACGCAGTACGTGTTCTGGGGTTTGATG
152	2266-2295	ACCACGGCATACCGACGCACGCAGTACGTG
201	3001-3030	TTCACCTGCAAATCCAGAAACAGGCTGTGA
202	3016-3045	ACCGTCTGGAGGCTGTTCACCTGCAAATCC
203	3031-3060	TAGATGTTGGTGCACACCGTCTGGAGGCTG
208	3106-3135	TTCCAAACTTGCTGATGAAATGGGAGCTGC
209	3121-3150	AAAAATGTGGGGTTCTTCCAAACTTGCTGA
210	3136-3165	GAGATGACGCGCAGGAAAAATGTGGGGTTC

PS-ODN#	Position (3'-5')	5'-AS sequence-3'
211	3151-3180	AGGGAGGCCGTGTCAGAGATGACGCGCAGG
212	3166-3195	AGGATGGAGTAGCAGAGGGAGGCCGTGTCA
213	3181-3210	GCGTTCTTGGCTTTCAGGATGGAGTAGCAG
230	3436-3465	GCGGGTGGCCATCAGTCCAGGATGGTCTTG
237	3541-3570	CAGACTCCCAGCGGTGCGGGCCTGGGTGTG
241	3601-3630	AGCCGGACACTCAGCCTTCAGCCGGACATG

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

EXAMPLES

The following examples are provided by way of illustration only and not by way of limitation. Those of skill will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

Example I: Inhibition of hTRT expression *in vitro*

In this example, inhibition of hTRT expression was examined using an *in vitro* cell-free expression system. A series of 30-mer antisense phosphorothioate oligonucleotides (PS-ODNs), which span the entire hTRT sequence, was systematically assayed for the ability to block hTRT expression *in vitro* (see Figure 3). Co-expression of luciferase was used to normalize the samples and demonstrate the specificity of inhibition.

For inhibition of hTRT expression *in vitro*, an hTRT transcription/expression plasmid was prepared according to standard methodology for *in vitro* transcription and translation of hTRT RNA. Coupled transcription-translation reactions were performed with a reticulocyte lysate system (Promega TNT™) according to standard conditions (as performed in Example 7, U.S. Patent application serial no. 08/974,549). Each coupled transcription/translation reaction included hTRT RNA transcribed from the expression plasmid, and a test antisense polynucleotide at a range of standard test concentrations, as well as the luciferase transcription/translation internal control (see, e.g., Sambrook et al., *supra*, Ausubel et al., *supra*). The translation reaction can also be performed with hTRT RNA that is synthesized *in vitro* in a separate reaction and then added to the translation reaction. ^{35}S-Met was included in the reaction to label the translation products. The negative control was performed without added PS-ODN.

The labeled translation products were separated by gel electrophoresis and quantitated after exposing the gel to a phosphorimager screen. The amount of hTRT protein expressed in the presence of hTRT specific PS-ODNs was normalized to the co-expressed luciferase control. The data are presented in Figure 3 as a percentage of the control, which is without added PS-ODN.

Example II: Inhibition of hTRT expression *ex vivo*

A. Reagents

Cells: ACHN cells, NCI, catalogue #503755; 293 cells, ATCC; BJ (see, e.g., Kim et al., *Science* 266: 2011-2015 (1994)); additional cells from the ATCC or NCI.

Media and solutions: RPMI 1640 medium, BioWhitaker; DMEM/M199 medium, BioWhitaker; EMEM, BioWhitaker; Fetal Bovine Serum, Summit (stored frozen at -20°C, stored thawed at 4°C); Trypsin-EDTA, GIBCO (catalogue #25300-054) (stored frozen at -20°C, stored thawed 4°C; Isoton II (stored at RT); DMSO (stored at RT); oligonucleotides (see Table 1 and Figure 3, stored in solution at -20°C); PBS (Ca^{++}/Mg^{++} free); TE; 10 mM Tris-HCL, pH 8.0; 1 mM EDTA.

To prepare oligonucleotide stocks: Dissolve oligonucleotide nucleotides (PS-ODNs) in the appropriate amount of TE to make a concentrated stock solution (1 - 20 mM).

B. Treatment of cells ex vivo with antisense hTRT oligonucleotides

1. For plating cells prior to oligonucleotide treatment, stock cultures of cells in log-phase growth (in T75 flask) were used. ACNH, 293, and BJ cells were used in this assay. The media was removed by aspiration, and the cells were rinsed with 2-5 ml of PBS. 1 ml of trypsin-EDTA was added to the cells, swirled to distribute, and incubated for 2 minutes. The trypsin was inactivated with 9 ml of media. The cells were gently triturated with media. 200 μL of the cells were then counted with a Coulter counter and diluted to the appropriate volume and number of cells per well.

2. For 6-well dishes, 1.1×10^5 cells total per well, 2 ml/well were added. The cells were allowed to settle 4-6 h prior to any treatment with oligonucleotides. The amount of cells can be scaled up or down proportionally for 12-well, 100 mm, or 150 mm dishes. For example, for 12-well dishes, use 4.6×10^4 cells in 2 ml media; for 100 mm dishes use 6×10^5 cells in 10 ml media; for 150 mm dishes use 1.7×10^6 cells in 35 ml media.

3. Oligonucleotides were diluted in media and fed to the cells at a range of standard test concentrations. Serial, sterile dilutions of the ODNs (see, e.g., Table 1) were prepared in sterile, filtered media for feeding the cells. The cells were treated in single, duplicate, or triplicate wells. Control wells were treated with TE diluted in media.

4. The cells were fed daily with freshly diluted PS-ODN-media by aspirating the media and then feeding with 2 ml of freshly diluted oligonucleotide in media.

5. When cells were near 70-80% confluent (3-4 days), the number of cells was determined per well. The media was removed by aspiration, and the cells were rinsed twice with 2 ml PBS. O.5 ml trypsin-EDTA was added to the cells, swirled, and incubated for 2 minutes. The cells were triturated gently with 2 ml media per well. 200 μL of cells were counted in a Coulter counter. If necessary, the cells are replated at 1.1 x 10^5 cells per well, 2 ml media per well, and fed with PS-ODN as described above.

6. Samples of the cells were also harvested for analysis of telomerase activity by TRAP activity. The cells can also be analyzed by isolating RNA and performing RT-PCR, by TRF measurement, or by telomere length measurement (see, e.g., Example section, U.S. Patent application serial no. 08/974,549 for assay protocols).

7. The cell population doublings (PDLs) were calculated for each timepoint according to the following formula. PDLs (P): Pn = Pn-1 + [((ln(Total # cells)) - (ln(# cells plated))/ln(2)]

8. Graph PDL vs. time (in days) for the full dose range of each PS-ODN as compared to control untreated cells.

9. Steps 2-8 were repeated for the desired duration (usually 2-4 weeks) or until cell growth was inhibited significantly.

10. Table 1 shows exemplary oligonucleotides that were tested using this assay, and which inhibited telomerase expression and activity by approximately 50% or more.

WHAT IS CLAIMED IS:

1. An isolated, synthetic, substantially pure, or recombinant polynucleotide having a sequence that is at least about ten nucleotides in length to at least about 100 nucleotides in length and comprising a sequence that is substantially complementary or substantially identical to a contiguous sequence of an hTRT nucleic acid that has the nucleotide sequence of Figure 1.

2. An isolated, synthetic, substantially pure, or recombinant polynucleotide having a sequence that is at least about ten nucleotides in length to at least about 100 nucleotides in length and comprising a sequence exactly complementary or identical to a contiguous sequence of a nucleic acid encoding the hTRT protein of Figure 2.

3. The polynucleotide of claim 2, wherein the nucleic acid encoding the hTRT protein has the nucleotide sequence of Figure 1.

4. The polynucleotide of claim 2 that is a DNA.

5. The polynucleotide of claim 2 that is an RNA.

6. The polynucleotide of claim 2 that comprises one or more non-naturally occurring, synthetic nucleotides.

7. The polynucleotide of claim 2 that is identical to said contiguous sequence of a nucleic acid encoding the hTRT protein of Figure 1.

8. The polynucleotide of claim 2 that is exactly complementary to said contiguous sequence of a nucleic acid encoding the hTRT protein of Figure 1.

9. The polynucleotide of claim 8 that is an antisense polynucleotide.

10. The antisense polynucleotide of claim 9 that is at least about 20 nucleotides in length to at least about 50 nucleotides in length.

11. The polynucleotide of claim 2, wherein the polynucleotide inhibits telomerase activity by at least about 50% in transformed cells *ex vivo*, as compared to control cells that are not treated with the polynucleotide.

12. The polynucleotide of claim 2, wherein the polynucleotide inhibits telomerase expression by at least about 50% *in vitro*, as compared to control expression reactions that lack the polynucleotide.

13. The polynucleotide of claim 2 selected from the group consisting of PS-ODN number 3, 4, 7, 8, 16, 21, 25, 26, 27, 28, 29, 33, 40, 41, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 62, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 80, 81, 82, 83, 84, 85, 86, 87, 88, 93, 94, 96, 100, 112, 114, 130, 143, 144, 151, 152, 201, 202, 203, 208, 209, 210, 211, 212, 213, 230, 237, and 241.

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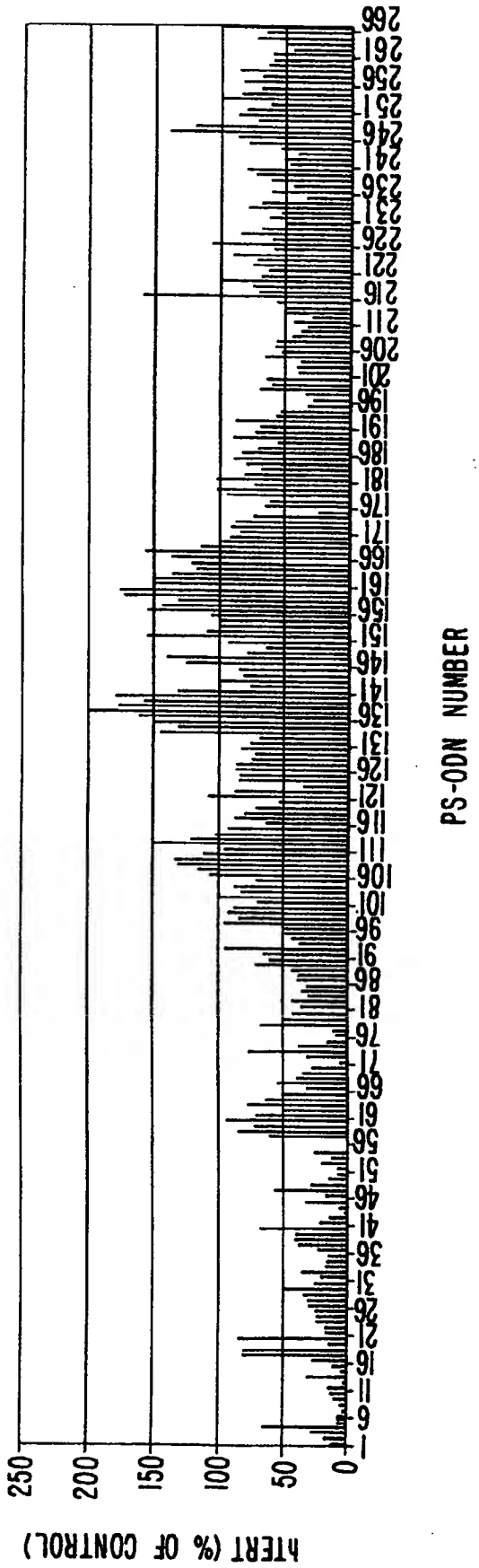
   1 GCAGCGCTGC GTCCTGCTGC GCACGTGGGA AGCCCTGGCC CCGGCCACCC CCGCGATGCC
  61 GCGCGCTCCC CGCTGCCGAG CCGTGCGCTC CCTGCTGCGC AGCCACTACC GCGAGGTGCT
 121 GCCGCTGGCC ACGTTCGTGC GGCGCCTGGG GCCCCAGGGC TGGCGGCTGG TGCAGCGCGG
 181 GGACCCGGCG GCTTTCCGCG CGCTGGTGGC CCAGTGCCTG GTGTGCGTGC CCTGGGACGC
 241 ACGGCCGCCC CCCGCCGCCC CCTCCTTCCG CCAGGTGTCC TGCCTGAAGG AGCTGGTGGC
 301 CCGAGTGCTG CAGAGGCTGT GCGAGCGCGG CGCGAAGAAC GTGCTGGCCT TCGGCTTCGC
 361 GCTGCTGGAC GGGGCCCGCG GGGGCCCCCC CGAGGCCTTC ACCACCAGCG TGCGCAGCTA
 421 CCTGCCCAAC ACGGTGACCG ACGCACTGCG GGGGAGCGGG GCGTGGGGGC TGCTGCTGCG
 481 CCGCGTGGGC GACGACGTGC TGGTTCACCT GCTGGCACGC TGCGCGCTCT TTGTGCTGGT
 541 GGCTCCCAGC TGCGCCTACC AGGTGTGCGG GCCGCCGCTG TACCAGCTCG GCGCTGCCAC
 601 TCAGGCCCGG CCCCCGCCAC ACGCTAGTGG ACCCCGAAGG CGTCTGGGAT GCGAACGGGC
 661 CTGGAACCAT AGCGTCAGGG AGGCCGGGGT CCCCCTGGGC CTGCCAGCCC CGGGTGCGAG
 721 GAGGCGCGGG GGCAGTGCCA GCCGAAGTCT GCCGTTGCCC AAGAGGCCCA GGCGTGGCGC
 781 TGCCCCTGAG CCGGAGCGGA CGCCCGTTGG GCAGGGGTCC TGGGCCCACC CGGGCAGGAC
 841 GCGTGGACCG AGTGACCGTG GTTTCTGTGT GGTGTCACCT GCCAGACCCG CCGAAGAAGC
 901 CACCTCTTTG GAGGGTGCGC TCTCTGGCAC GCGCCACTCC CACCCATCCG TGGGCCGCCA
 961 GCACCACGCG GGCCCCCCAT CCACATCGCG GCCACCACGT CCCTGGGACA CGCCTTGTCC
1021 CCCGGTGTAC GCCGAGACCA AGCACTTCCT CTACTCCTCA GGCGACAAGG AGCAGCTGCG
1081 GCCCTCCTTC CTACTCAGCT CTCTGAGGCC CAGCCTGACT GGCGCTCGGA GGCTCGTGGA
1141 GACCATCTTT CTGGGTTCCA GGCCCTGGAT GCCAGGGACT CCCCGCAGGT TGCCCCGCCT
1201 GCCCCAGCGC TACTGGCAAA TGCGGCCCCT GTTTCTGGAG CTGCTTGGGA ACCACGCGCA
1261 GTGCCCCTAC GGGGTGCTCC TCAAGACGCA CTGCCCGCTG CGAGCTGCGG TCACCCCAGC
1321 AGCCGGTGTC TGTGCCCGGG AGAAGCCCCA GGGCTCTGTG GCGGCCCCCG AGGAGGAGGA
1381 CACAGACCCC CGTCGCCTGG TGCAGCTGCT CCGCCAGCAC AGCAGCCCCT GGCAGGTGTA
1441 CGGCTTCGTG CGGGCCTGCC TGCGCCGGCT GGTGCCCCCA GGCCTCTGGG GCTCCAGGCA
1501 CAACGAACGC CGCTTCCTCA GGAACACCAA GAAGTTCATC TCCCTGGGGA AGCATGCCAA
1561 GCTCTCGCTG CAGGAGCTGA CGTGGAAGAT GAGCGTGCGG GACTGCGCTT GGCTGCGCAG
1621 GAGCCCAGGG GTTGGCTGTG TTCCGGCCGC AGAGCACCGT CTGCGTGAGG AGATCCTGGC
1681 CAAGTTCCTG CACTGGCTGA TGAGTGTGTA CGTCGTCGAG CTGCTCAGGT CTTTCTTTTA
1741 TGTCACGGAG ACCACGTTTC AAAAGAACAG GCTCTTTTTC TACCGGAAGA GTGTCTGGAG
1801 CAAGTTGCAA AGCATTGGAA TCAGACAGCA CTTGAAGAGG GTGCAGCTGC GGGAGCTGTC
1861 GGAAGCAGAG GTCAGGCACC ATCGGGAAGC CAGGCCCGCC CTGCTGACGT CCAGACTCCG
1921 CTTCATCCCC AAGCCTGACG GGCTGCGGCC GATTGTGAAC ATGGACTACG TCGTGGGAGC
1981 CAGAACGTTC CGCAGAGAAA AGAGGGCCGA GCGTCTCACC TCGAGGGTGA AGGCACTGTT
2041 CAGCGTGCTC AACTACGAGC GGGCGCGGCG CCCCGGCCTC CTGGGCGCCT CTGTGCTGGG
2101 CCTGGACGAT ATCCACAGGG CCTGGCGCAC CTTCGTGCTG CGTGTGCGGG CCCAGGACCC
2161 GCCGCCTGAG CTGTACTTTG TCAAGGTGGA TGTGACGGGC GCGTACGACA CCATCCCCCA
2221 GGACAGGCTC ACGGAGGTCA TCGCCAGCAT CATCAAACCC CAGAACACGT ACTGCGTGCG
2281 TCGGTATGCC GTGGTCCAGA AGGCCGCCCA TGGGCACGTC CGCAAGGCCT TCAAGAGCCA
2341 CGTCTCTACC TTGACAGACC TCCAGCCGTA CATGCGACAG TTCGTGGCTC ACCTGCAGGA
2401 GACCAGCCCG CTGAGGGATG CCGTCGTCAT CGAGCAGAGC TCCTCCCTGA ATGAGGCCAG
2461 CAGTGGCCTC TTCGACGTCT TCCTACGCTT CATGTGCCAC CACGCCGTGC GCATCAGGGG
2521 CAAGTCCTAC GTCCAGTGCC AGGGGATCCC GCAGGGCTCC ATCCTCTCCA CGCTGCTCTG
2581 CAGCCTGTGC TACGGCGACA TGGAGAACAA GCTGTTTGCG GGGATTCGGC GGGACGGGCT
2641 GCTCCTGCGT TTGGTGGATG ATTTCTTGTT GGTGACACCT CACCTCACCC ACGCGAAAAC
2701 CTTCCTCAGG ACCCTGGTCC GAGGTGTCCC TGAGTATGGC TGCGTGGTGA ACTTGCGGAA
2761 GACAGTGGTG AACTTCCCTG TAGAAGACGA GGCCCTGGGT GGCACGGCTT TTGTTCAGAT
2821 GCCGGCCCAC GGCCTATTCC CCTGGTGCGG CCTGCTGCTG GATACCCGGA CCCTGGAGGT
2881 GCAGAGCGAC TACTCCAGCT ATGCCCGGAC CTCCATCAGA GCCAGTCTCA CCTTCAACCG
2941 CGGCTTCAAG GCTGGGAGGA ACATGCGTCG CAAACTCTTT GGGGTCTTGC GGCTGAAGTG
3001 TCACAGCCTG TTTCTGGATT TGCAGGTGAA CAGCCTCCAG ACGGTGTGCA CCAACATCTA
3061 CAAGATCCTC CTGCTGCAGG CGTACAGGTT TCACGCATGT GTGCTGCAGC TCCCATTTCA
3121 TCAGCAAGTT TGGAAGAACC CCACATTTTT CCTGCGCGTC ATCTCTGACA CGGCCTCCCT
3181 CTGCTACTCC ATCCTGAAAG CCAAGAACGC AGGGATGTCG CTGGGGGCCA AGGGCGCCGC
3241 CGGCCCTCTG CCCTCCGAGG CCGTGCAGTG GCTGTGCCAC CAAGCATTCC TGCTCAAGCT
3301 GACTCGACAC CGTGTCACCT ACGTGCCACT CCTGGGGTCA CTCAGGACAG CCCAGACGCA
3361 GCTGAGTCGG AAGCTCCCGG GGACGACGCT GACTGCCCTG GAGGCCGCAG CCAACCCGGC
3421 ACTGCCCTCA GACTTCAAGA CCATCCTGGA CTGATGGCCA CCCGCCCACA GCCAGGCCGA
3481 GAGCAGACAC CAGCAGCCCT GTCACGCCGG GCTCTACGTC CCAGGGAGGG AGGGGCGGCC
3541 CACACCCAGG CCCGCACCGC TGGGAGTCTG AGGCCTGAGT GAGTGTTTGG CCGAGGCCTG
3601 CATGTCCGGC TGAAGGCTGA GTGTCCGGCT GAGGCCTGAG CGAGTGTCCA GCCAAGGGCT
3661 GAGTGTCCAG CACACCTGCC GTCTTCACTT CCCCACAGGC TGGCGCTCGG CTCCACCCCA
3721 GGGCCAGCTT TTCCTCACCA GGAGCCCGGC TTCCACTCCC CACATAGGAA TAGTCCATCC
3781 CCAGATTCGC CATTGTTCAC CCCTCGCCCT GCCCTCCTTT GCCTTCCACC CCCACCATCC
3841 AGGTGGAGAC CCTGAGAAGG ACCCTGGGAG CTCTGGGAAT TTGGAGTGAC CAAAGGTGTG
3901 CCCTGTACAC AGGCGAGGAC CCTGCACCTG GATGGGGGTC CCTGTGGGTC AAATTGGGGG
3961 GAGGTGCTGT GGGAGTAAAA TACTGAATAT ATGAGTTTTT CAGTTTTGAA AAAAA
```

FIG. 1.

MPRAPRCRAVRSLLRSHYREVLPLATFVRRLGPQGWRLVQRGDPAAFRALVAQCLVCV
PWDARPPPAAPSFRQVSCLKELVARVLQRLCERGAKNVLAFGFALLDGARGGPPEAFT
TSVRSYLPNTVTDALRGSGAWGLLLRRVGDDVLVHLLARCALFVLVAPSCAYQVCGPP
LYQLGAATQARPPPHASGPRRRLGCERAWNHSVREAGVPLGLPAPGARRRGGSASRSL
PLPKRPRRGAAPEPERTPVGQGSWAHPGRTRGPSDRGFCVVSPARPAEEATSLEGALS
GTRHSHPSVGRQHHAGPPSTSRPPRPWDTPCPPVYAETKHFLYSSGDKEQLRPSFLLS
SLRPSLTGARRLVETIFLGSRPWMPGTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYG
VLLKTHCPLRAAVTPAAGVCAREKPQGSVAAPEEEDTDPRRLVQLLRQHSSPWQVYGF
VRACLRRLVPPGLWGSRHNERRFLRNTKKFISLGKHAKLSLQELTWKMSVRDCAWLRR
SPGVGCVPAAEHRLREEILAKFLHWLMSVYVVELLRSFFYVTETTFQKNRLFFYRKSV
WSKLQSIGIRQHLKRVQLRELSEAEVRQHREARPALLTSRLRFIPKPDGLRPIVNMDY
VVGARTFRREKRAERLTSRVKALFSVLNYERARRPGLLGASVLGLDDIHRAWRTFVLR
VRAQDPPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRRYAVVQKAAHGH
VRKAFKSHVSTLTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSGLFDVFLRF
MCHHAVRIRGKSYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRDGLLLRLVDDF
LLVTPHLTHAKTFLRTLVRGVPEYGCVVNLRKTVVNFPVEDEALGGTAFVQMPAHGLF
PWCGLLLDTRTLEVQSDYSSYARTSIRASLTFNRGFKAGRNMRRKLFGVLRLKCHSLF
LDLQVNSLQTVCTNIYKILLLQAYRFHACVLQLPFHQQVWKNPTFFLRVISDTASLCY
SILKAKNAGMSLGAKGAAGPLPSEAVQWLCHQAFLLKLTRHRVTYVPLLGSLRTAQTQ
LSRKLPGTTLTALEAAANPALPSDFKTILD

FIG. 2.


PS-ODN NUMBER
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196
201
206
211
216
221
226
231
236
241
246
251
256
261
266
0
50
100
150
200
250
hTERT (% OF CONTROL)

FIG. 3.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/07160

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/04; A01N 43/04; A61K 31/70, 38/00
US CL : 536/23.5; 514/44; 530/324
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 514/44; 530/324

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NAKAMURA et al. "Telomerase Catalytic Subunit Homologs from Fission Yeast and Human" Science. 15 August 1997. Vol. 277. No. 5328. pages 955-959, especially page 957.	1-12
X	MEYERSON et al. "hEST2, the Putative Human Telomerase Catalytic Subunit Gene, is up-regulated in Tumor Cells and During Immortalization" Cell. 22 August 1997. Vol. 90. No. 4. pages 785-795, especially page 787.	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 AUGUST 1999	Date of mailing of the international search report 30 AUG 1999
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Form PCT/ISA/210 (second sheet)(July 1992)*